BEHAVIORAL AND NEURAL BIOLOGY 39, 299–306 (1983)

BRIEF REPORT

Degranulation of Brain Mast Cells in Young Albino Rats

MICHAEL A. PERSINGER[1]

Neuroscience Laboratory, Department of Psychology, Laurentian University, Sudbury, Ontario P3E 2C6, Canada

Quantitative measurements were made of brain mast cell (MC) degranulation in juvenile albino rats. Neither acute nor chronic intraperitoneal injections of Compound 48/80 (C 48/80) evoked any clear degranulation. Fifteen to thirty minutes after the injection of either C 48/80 or physiological saline into the right anterior thalamus, frank degranulation in the leptomeninges and some degranulation in the parenchyma were evident. The injected sides contained about twice as much degranulated cells as the noninjected sides. In a second experiment, animals were killed 10, 100, 1000, and 10,000 min after a single 10-μl injection of C 48/80 into the anterior thalamus. Although about 40% of the leptomeningeal and 20% of the parenchymal MCs were degranulated within 10 min, more than 50% of the MCs in both areas were degranulated within 100 min after the injection. More extensive degranulation was evident at both times within the parenchyma of the injected sides. Degranulated MCs were not obvious after this period although nuclei surrounded by sparse granules (in the parenchyma) or by fused, globular metachromatic material (in the leptomeninges or their ventricular processes) were still discernable 7 days later. The implications of brain mast cell degranulation for psychoneuroimmunology are considered.

Body mast cells (MCs) are notorious for their explosive degranulation that can release, within minutes, histamine, serotonin (in some species), heparin, and complex proteins into the extracellular fluid (Selye, 1965). The capacity for *brain* MCs to quickly degranulate in response to the appropriate stimulus, could have drastic consequences (Persinger, 1977a). Most brain mast cells are found in the periventricular organs (in man) or within the leptomeninges and around thalamic blood vessels (in the rat).

Degranulation of a cluster of MCs or even a single MC would markedly modify the local extracellular fluid or blood brain barrier. The potential contribution of MC function to the development of neuroimmunological disorders has been implied by both clinical and experimental studies.

[1] Thanks to R. M. Krema and to Gyslajne Lafrenière for technical assistance.

0163-1047/83 $3.00
Copyright © 1983 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Olsson (1974) has repeatedly noted the presence of MCs around multiple sclerotic plaques. Recently, Mokhtarien and Griffin (1982) reported a reduced encephalitic response in mice infected with Sindbis virus following injections of reserpine (a substance that seems to deplete serotonin levels in MCs). Presumably the normal response of mononuclear inflammation is dependent upon T-cell mediated release of serotonin by mast cells.

That brain MCs may be a "gateway" by which sensitized lymphocytes enter brain parenchyma (Persinger, 1977a) is an intriguing although frightening speculation. In this laboratory, one excellent example of clearly aberrant infiltrations of mononuclear (lymphocyte-like) cells within the anterior thalamus (anteroventral nucleus) of a naive 20-day old Wistar rat has been documented. The side containing the lesion is devoid of mast cells while the normal side contains typical MC numbers.

Direct lipolytic consequences of MC degranulation are also a strong possibility in brain space. Baloyannis and Theoharides (1982) recently found that brain slices exposed to media enriched with MCs collected from the peritoneal cavity or MCs plus Compound 48/80 (C 48/80) demonstrated extensive demyelination. Pronounced accumulation of MCs were noted around the demyelinated areas. Brain sections exposed to media enriched with only C 48/80, a supernatant fluid from MC suspensions, or control conditions demonstrated normal myelin development.

These observations have significant behavioral implications. Whereas about only 20% of the total diencephalic MCs are prominent within the parenchyma in 15-day-old albino rat brains, more than 50% of the total MCs occur (primarily around blood vessels) within the thalamus of 20-day-old brains (Persinger, 1980). If the MCs that appear in the thalamic parenchyma of the 20-day-old rat brain are related to the MCs that "disappear" from the leptomeninges of the 15-day-old brain (Persinger, 1981), one obvious question follows. Do these cells proceed through labile stages during which time they could *degranulate,* following appropriate neuronal inputs or chemical alterations?

Two separate experiments were completed to answer this question. Animal care procedures and environmental conditions have been reported elsewhere (Persinger, 1980). In Experiment I, 37, 20- to 21-day-old albino Wistar rats (*Rattus norvegicus*) from five different litters were used as subjects. The experimental treatments involved four major groups: (1) chronic ip (intraperitoneal) injections of either C 48/80 or saline; (2) acute ip injections of C 48/80 (with two subgroups killed either 30 min or 3 hr later); (3) direct injections of either C 48/80 or saline into the right anterior thalamus; and (4) noninjected controls. Litters and sex were counterbalanced with treatments.

The dosage of each rat in the chronic C 48/80 group was increased (two injections/day; once every 12 hours) in daily increments of 40 μg from 40 μg to 200 μg (1 mg/kg to 5 mg/kg) over 5 consecutive days.

Comparable schedules for adult rats allegedly degranulate all body MCs (Heap & Kiernan, 1973). Subjects in the acute injection group received a single injection of 5 mg/kg of C 48/80 (larger dosage produced mortality). Following 0.03 cc of 50 mg/cc sodium pentobarbital, intrathalamic rats received between 10 and 50 μl of 5 mg/cc C 48/80 or saline through a 50-μl syringe at the level of the right anteroventral thalamus. All the latter animals were killed within 15 to 30 min after the injections. The brains of two rats from different litters were severely distorted (sandwiched between two slides by 30*g* for 1 min) to determine any crude mechanical effects.

In Experiment II, 18, 20-day-old Wistar Albino pups from three litters whose parents had been obtained from Woodlyn Laboratories, Guelph, Ontario, were selected. After surgical anaesthesia (1.6 mg of sodium pentobarbital in 0.1 cc saline: 0.025 cc Somnitol plus 0.075 cc isotonic saline), each rat was injected (within 15–20 sec, using a 50-μl Hamilton syringe) with 10 μl of 1 mg/cc C 48/80 (in isotonic saline) within the right anterior thalamus. Control animals did not receive any injections. One thalamic-injected animal from each litter was killed 10, 100, 1000, or 10,000 minutes after the injection; control pups were killed at the latter two periods.

All brains were fixed in E.F.A. and processed routinely (Persinger, 1981, 1980). Either five (Experiment I) or nine (Experiment II) 10-μm coronal sections equally spaced between the caudal and rostral thalamic boundaries were selected from each animal. *Each* MC (Persinger, 1981) as defined by both metachromasia and morphology, was classified according to the following scheme: Class I (a typical MC characterized by deep, complete purplish staining with no or few granules outside the membrane); Class II (either more than 10 granules outside the membrane or sparser granule distribution within the cytoplasm—these two characteristics usually occurred together); and Class III (no clear cell boundary, obvious and massive degranulation with metachromatic granules dispersed in an area 4 to 16 times larger than the typical cytoplasmic area.) The latter is rarely seen in normal brains.

To determine any "injection effects," the left and right sides of the thalamus were classified separately. Due to the possible reactive differences between leptomeningeal and parenchymal MCs, these two populations were classified separately as well. In Experiment II, the appearance of two other metachromatic classifications were required. Class A (clear nuclei surrounded by large undifferentiated metachromatic masses and Class B (nuclei with shapes typical of normal MCs but with smaller cytoplasmic areas and very few metachromatic granules). All statistical analyses were completed by SPSS software on a DEC 2020 System computer.

Histological inspection indicated that all thalamic injection sites were

at the level of the anteroventral nuclei and penetrated not further then their most ventral portion. The means and standard deviations for the numbers of MCs/section and the percentages of Class I, II, and III MCs for the leptomeninges and parenchyma are shown in Tables 1 and 2. One way analysis of variance indicated that treatment differences explained 50% (ω^2) of the variance in percentages of Class III MCs for either side (left, $F = 12.04$; right, $F(3, 31) = 11.65$; $p < .001$) of the leptomeninges. There was no significant difference between groups for the percentages of Class III MCs in the left parenchyma ($F < 1$; $\varsigma^2 = 4\%$) but a significant ($p < .01$) difference within the injected parenchyma ($F = 10.19$; $\omega^2 = 49\%$).

TABLE 1
Means and Standard Deviations for Numbers of MCs/10-μm Section and for Relative Percentages of the Three Classes of MCs in the Left and Right Diencephalic Leptomeninges as a Function of the Four Major Experimental Manipulations

	Thalamic leptomeninges			
		Percentages		
Group	Numbers (MCs/10 μm)	Class I	Class II	Class III
		Left side		
Control ($n = 5$)	10.5 ± 10.9	91 ± 7[a]	8 ± 7	1 ± 1[b]
Intrathalamic ($n = 10$)	9.2 ± 7.8	48 ± 24[b]	29 ± 23	23 ± 15[a]
Acute ip ($n = 10$)	6.4 ± 3.2	76 ± 25[a]	23 ± 20	1 ± 4[b]
Chronic ip ($n = 10$)	6.1 ± 3.6	89 + 17[a]	10 ± 14	1 ± 4[b]
"Squashed" ($n = 2$)	8.9 ± 5.5	68 ± 6	27 ± 10	6 ± 4
		Right side		
Control ($n = 5$)	8.7 ± 7.1	90 ± 2[a]	9 ± 3[a]	2 ± 2
Intrathalamic ($n = 10$)	6.1 ± 4.6	26 ± 21[b]	33 ± 22[b]	42 ± 32
Acute ip ($n = 10$)	6.0 ± 3.7	85 ± 14[a]	14 ± 14[a]	1 ± 1
Chronic ip ($n = 10$)	5.5 ± 3.7	95 ± 5[a]	6 ± 5[a]	0 ± 0
"Squashed" ($n = 2$)	5.0 ± 0.3	74 ± 1	24 ± 1	2 ± 2

Note. The results of two brains that had been deformed severely ("squashed") are shown for comparison but were not included in the statistical analyses.

[a,b] *a* vs *b* (for columns only), $p \leq .05$ according to ad hoc tests (Scheffe's) following ANOVAs.

TABLE 2
Means and Standard Deviations for Numbers of MCs/10-μm Section and for Relative Percentages of the Three Classes of MCs in the Left and Right Thalamic Parenchyma as a Function of the Four Major Experimental Manipulations

	Thalamic parenchyma			
		Percentages		
Group	Numbers (MCs/10 μm)	Class I	Class II	Class III
		Left side		
Control (n = 5)	10.4 ± 4.5	81 ± 6[a]	18 ± 4[a]	2 ± 3
Intrathalamic (n = 10)	6.4 ± 4.2	68 ± 17[b]	26 ± 10	6 ± 10
Acute ip (n = 10)	12.5 ± 10.8	53 ± 13[b]	41 ± 12[b]	6 ± 6
Chronic ip (n = 10)	10.5 ± 7.5	66 ± 12[b]	31 ± 13	3 ± 3
"Squashed" (n = 2)	4.9 ± 2.4	56 ± 1	45 ± 1	0 ± 0
		Right side		
Control (n = 5)	6.5 ± 2.3	81 ± 5[a]	18 ± 4[a]	1 ± 1[a]
Intrathalamic (n = 10)	5.8 ± 3.4	42 ± 20[b]	36 ± 12[b]	21 ± 16[b]
Acute ip (n = 10)	13.5 ± 13.6	55 ± 8[b]	42 ± 10[b]	3 ± 3[a]
Chronic ip (n = 10)	7.3 ± 4.7	76 ± 13	23 ± 14[a]	1 ± 1[a]
"Squashed" (n = 2)	10.1 ± 10.0	68 + 8	28 ± 11	5 ± 3

[a,b] a vs b (for columns only), $p \leq .05$ according to ad hoc (Scheffe's) test following ANOVAs.

Post hoc tests (Scheffes, $p < .05$) indicated that the effect was due to the differences between the intrathalamically injected rats compared to the other three treatments. Non-parametric tests (Kruskal–Wallis) confirmed this relationship. Multiple regression analyses demonstrated that the percentages of Class III MCs were influenced by simple injection volume within the left leptomeninges ($r = +0.60$) and the right parenchyma ($r = +0.45$) only but not by either kill latency, sex, or body weight. Litter differences explained about 45% of the variance in MC numbers which were not affected by any of the treatments.

The means and standard deviations for the numbers of MCs/10-μm section and for the percentages of different MC classes for Experiment II are shown in Tables 3 and 4. Since brains of rats killed 10 and 100 min after injection were qualitatively different (no Class A and B cells)

TABLE 3
Means and Standard Deviations for the Relative Percentages of the Five Classes of MCs and Numbers of MCs/10-μm Section in the Left and Right Thalamic Leptomeninges as a Function of Time (Log of the Minutes) since Injection or Control Treatment

Log of Time (min)	A	B	No. of MCs/10 μm	Class I	Class II	Class III
			Left side injected			
1	0 ± 0	0 ± 0	7.9 ± 4.3	35 ± 23	22 ± 19	43 ± 7
2	10 ± 5	2 ± 2	5.1 ± 0.4	13 ± 9	9 ± 5	65 ± 21
3	27 ± 27	28 ± 11	1.4 ± 1.0	38 ± 27	8 ± 7	0 ± 0
4	44 ± 32	3 ± 3	1.0 ± 0.4	37 ± 29	14 ± 9	2 ± 3
			Left side control			
3	0 ± 0	0 ± 0	3.5 ± 2.8	82 ± 21	17 ± 21	0 ± 0
4	0 ± 0	0 ± 0	0.2 ± 0.1	100 ± 0	0 ± 0	0 ± 0
			Right side injected			
1	0 ± 0	0 ± 0	5.7 ± 2.6	26 ± 3	34 ± 3	40 ± 4
2	4 ± 7	7 ± 6	3.8 ± 1.1	7 ± 2	28 ± 21	53 ± 21
3	59 ± 5	30 ± 8	0.4 ± 0.4	9 ± 9	0 ± 0	1 ± 1
4	68 ± 22	5 ± 5	0.7 ± 0.7	19 ± 15	7 ± 12	0 ± 0
			Right side control			
3	0 ± 0	1 ± 2	2.5 ± 2.1	87 ± 8	11 ± 15	0 ± 0
4	0 ± 0	0 ± 0	1.0 ± 0.4	86 ± 10	14 ± 14	0 ± 0

than those killed at 1K min or 10K min later (no Class III cells), treatments were combined into three groups: (1) 10 min + 100 min; (2) 1 kmin + 10 kmin); and (3) controls.

According to one-way ANOVAs (and reaffirmed by Kruskal–Wallis tests), Group 1 had significantly ($p < .001$) more Class III cells within the leptomeninges ($F(2, 15) = 48.65$; $\chi^2 = 14.84$) and parenchyma ($F(2, 15) = 18.78$; $\chi^2 = 12.50$) than the other two groups. Correlated t tests indicated that only the parenchyma demonstrated significantly higher percentages of Class III MCs on the injected side relative to the other.

Group 2 (1K-min plus 10K-min kills) contained significantly more percentages of Class A cells in the left ($F = 5.79$; $\chi^2 = 9.05$) and right ($F = 92.07$; $\chi^2 = 14.84$) leptomeninges compared to the other two groups and the right injected side contained more than the left (correlated $t(5) = 3.80$; $p < .05$). Group 2 also demonstrated a significant elevation of the percentage of Class B cells within both the left and right ($F = 3.82$; $\chi^2 = 9.87$) parenchyma compared to the other groups; there were no side differences or leptomeningeal effects. There were no significant differences in numbers of MCs between any of the groups.

This study clearly indicates that various schedules of ip injections of C 48/80 do not significantly alter either brain MC numbers or (light microscopic) morphology. Injections of small volumes of C 48/80 directly

TABLE 4
Means and Standard Deviations for the Relative Percentages of the Five Classes of MCs and Numbers of MCs/10-μm Section in the Left and Right Thalamic Parenchyma as a Function of Time (Log of Minutes) since Injection or Control Procedures

Log of time (min)	A	B	No. of MCs/10 μm	Class I	Class II	Class III
			Left side injected			
1	0 ± 0	3 ± 2	5.8 ± 1.0	51 ± 18	27 ± 12	18 ± 7
2	3 ± 3	3 ± 1	7.5 ± 3.8	28 ± 10	27 ± 7	39 ± 14
3	5 ± 5	22 ± 11	1.0 ± 0.3	53 ± 26	14 ± 14	5 ± 8
4	0 ± 0	4 ± 4	5.0 ± 5.1	47 ± 17	38 ± 8	10 ± 15
			Left side control			
3	1 ± 1	6 ± 6	3.1 ± 3.0	75 ± 29	18 ± 21	1 ± 2
4	0 ± 0	3 ± 5	4.2 ± 0.5	54 ± 7	37 ± 2	6 ± 1
			Right side injected			
1	0 ± 0	0 ± 0	3.8 ± 1.5	44 ± 16	23 ± 7	33 ± 15
2	0 ± 0	2 ± 1	4.6 ± 0.9	27 ± 22	12 ± 9	59 ± 30
3	8 ± 14	54 ± 11	0.3 ± 0.4	37 ± 15	0 ± 0	0 ± 0
4	0 ± 0	16 ± 14	7.1 ± 5.0	52 ± 17	30 ± 7	1 ± 1
			Right side control			
3	0 ± 0	28 ± 40	6.1 ± 5.8	54 ± 29	16 ± 21	1 ± 2
4	0 ± 0	3 ± 4	5.8 ± 1.7	66 ± 8	29 ± 4	1 ± 1

into the MC rich anterior ventral thalamic nuclei evoke side- and time-dependent increases in MC degranulation. These cells were not evident within 24 hr of the injection, although cells containing metachromatic materials (Class A and Class B cells), reminiscent of regranulating nonneural MCs (Persinger and Fiss, 1978), appeared. Class B morphologies are also evident in normal animals just before the precipitous age-dependent drop in MC numbers (Persinger, 1981).

The importance of MC degranulation due to behavioral treatments (Persinger, 1980, 1977b) has not been addressed. Few researchers have sensitized young animals to parenchymal tissue or have closely studied the cytomorphology in MC areas of the young rat brain. Massive degranulation, of the type noted in this study, may not be required for a significant neurobiological consequence. Assuming 10 pg of histamine (or serotonin) per cell, the sudden release of a *single* MC could produce a microenvironment of 0.01 *M* histamine within the immediate extracellular space. The resulting microleakage around the vasculature could be a gateway by which sensitized cells could contact parenchyma.

REFERENCES

Baloyannis, S. J., & Theoharides, T. C. (1982). Mast cells may induce demyelination of rat brain slices in vitro. *Clinical Research,* **30,** 407A.

Heap, B. J., and Kiernan, J. A. (1973). Histological, histochemical and pharmacological observations in mast cells in the stomach of the rat. *Journal of Anatomy*, **115,** 315–325.

Mokhtarian, F., & Griffin, D. E. (1982). The role of mast cells in the CNS inflammatory response of mice with Sindbis Virus Encephalitis. *Federation Proceedings*, **41,** 557.

Olsson, Y. (1974). Mast cells in plaques of multiple sclerosis. *Acta Neurologica Scandinavica*, **50,** 611–618.

Persinger, M. A. (1977). Mast cells in the brain: Possibilities for physiological psychology. *Physiological Psychology*, **5,** 166–176. (a)

Persinger, M. A. (1977). Preweaning body marking reduces brain mast cell numbers in rats. *Behavioral Biology*, **21,** 426–431. (b)

Persinger, M. A. (1980). Handling factors not body marking influence thalamic mast cell numbers in the preweaned albino rat. *Behavioral and Neural Biology*, **30,** 448–459.

Persinger, M. A. (1981). Developmental alterations in mast cell numbers and distributions within the thalamus of the albino rat. *Developmental Neuroscience* **4,** 220–224.

Persinger, M. A., & Fiss, T. B. (1978). Mesenteric mast cell degranulation is not essential for conditioned taste aversion. *Pharmacology, Biochemistry and Behavior*, **9,** 725–730.

Selye, H. (1965). *The Mast Cells*. Washington: Buttersworth.